

## Microbiological Hazards of Household Toilets: Droplet Production and the Fate of Residual Organisms

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Large numbers of bacteria and viruses when seeded into household toilets were shown to remain in the bowl after flushing, and even continual flushing could not remove a persistent fraction. This was found to be due to the adsorption of the organisms to the porcelain surfaces of the bowl, with gradual elution occurring after each flush. Droplets produced by flushing toilets were found to harbor both bacteria and viruses which had been seeded. The detection of bacteria and viruses falling out onto surfaces in bathrooms after flushing indicated that they remain airborne long enough to settle on surfaces throughout the bathroom. Thus, there is a possibility that a person may acquire an infection from an aerosol produced by a toilet.

The transmission of disease by aerosols from toilets has received only limited study. It has been suggested that, aside from coughing and sneezing, this must be the most common process involved in the generation of infectious aerosols (6). Darlow and Bale (6) demonstrated the production of bacterial aerosols, with the aid of both a liquid impinger and a slit sampler, from flushed bowls containing *Serratia marcescens*. These aerosols were found to persist for at least 12 min after the flush. The generation of aerosols by toilets seeded with coliform bacteria has been demonstrated by Bound and Atkinson (3) and more recently by Newsom (14). The size of particles produced by the flushing toilet was found to be in the range that was capable of reaching the lower respiratory tract (6). In addition, pathogenic fecal contaminants, such as *Escherichia* and *Salmonella*, have been isolated from the respiratory tract of infected humans (6).

The fallout of droplets containing pathogens on bathroom surfaces is also of concern, since hand contact with contaminated surfaces can result in self-inoculation by touching of the nose or mouth (11). Hutchinson (12) traced the spread of *Shigella sonnei* in a nursery school to contaminated toilet surfaces. Contact with contaminated surfaces has also been shown to be important in the spread of animal viruses (11).

To date no information exists on the generation of viral aerosols by household toilets. This study was carried out to gather more information on the fate of both bacteria and viruses in flushed toilets.

### MATERIALS AND METHODS

**Viruses and virus assays.** *E. coli* bacteriophage MS-2 and a plaque-purified line of type 1 poliovirus (strain LSc) were used in this study. MS-2, like poliovirus, is a small (25-nm diameter) icosahedral ribonucleic acid virus. All bacteriophage assays were done by a modification of the agar overlay method as described by Adams (1). Overlay agar and broth used for bacteriophage samples were prepared according to Davis and Sinsheimer (8). Stock poliovirus was grown in baboon kidney cells, concentrated 10-fold, and partially purified by membrane chromatography (22). Poliovirus samples were diluted in tris(hydroxymethyl)aminomethane-buffered saline containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Poliovirus assays were made with BSC-1 cells by the plaque-forming unit method as used in this laboratory (20).

**Bacteria and bacterial assays.** A strain of *E. coli* isolated from domestic sewage was used (identification based on ImVic test). All coliform assays were performed on Levine eosin methylene blue (EMB) agar. Total aerobic bacterial counts were performed on Standard Methods agar (BBL, Cockeysville, Md.). Cultures of *E. coli* used in seeding experiments were grown overnight in Trypticase soy broth (TSB) (BBL, Cockeysville, Md.). All bacterial samples were diluted in tris(hydroxymethyl)aminomethane-buffered saline.

**Toilets.** Standard household tank or valve toilets were used. The tank toilets had a reservoir containing approximately 20 liters, of which 13.7 liters was released during a flush, unless otherwise noted. The bowl of the tank-type toilet contained a volume of 3.5 liters unless indicated otherwise. The bowl volume of the valve toilet was approximately 4.2 liters, with a standard but undetermined amount of water released during a flush. In the valve toilet used, the

amount of water released was dependent on the water line pressure in the building, which was usually constant. Before being seeded with bacteria or viruses the toilets were cleaned with commercially available chlorine-containing cleanser and flushed repeatedly to eliminate any bacteria or viruses naturally present. A solution of 5 g of sodium thiosulfate per liter was then added to inactivate any chlorine present in the water, at a ratio of 1 ml of solution to 1 liter of tapwater. A tank-type toilet was used in all experiments unless indicated otherwise.

## RESULTS

**Residual infectious material in toilet bowls.** The first group of experiments was conducted to determine the fate of infectious agents in toilet waters after flushing of a typical domestic toilet. Toilet bowls were first cleaned as described in Materials and Methods and then seeded with either an overnight culture of *E. coli* or MS-2 phage. In both cases the organisms were suspended in 100 ml of TSB to simulate the presence of organic matter found in actual fecal material. After the bowl water was mixed, a baseline sample was obtained. The toilet was then flushed, and the toilet water was sampled for residual organisms. This procedure was repeated several times, and the results of a typical experiment for both bacteria and viruses can be seen in Fig. 1. As anticipated, the initial flush eliminated the major proportion of exogenously added organisms. However, after repeated flushes, instead of diminishing, there was often an increase in the number of residual organisms detected in the bowl. In the case of both bacteria and viruses, the number of organisms in the bowl reached a plateau below which their number could not be reduced, even after repeated flushing. From this evidence, it appeared that significant numbers of bacteria and viruses were being adsorbed to the toilet porcelain and then eluted during the flushing action.

To test this speculation, the previous experiment was repeated, and, after the third flush, Tween 80, a nonionic detergent, was added to yield a final concentration of 0.1% in the bowl water. The sides of the bowl were then scrubbed with a brush, and a sample was obtained for assay. The results of this experiment for *E. coli* are shown in Fig. 2. A 10,000-fold increase in the bacterial count occurred after the Tween 80 treatment, indicating that bacteria were adsorbing to the porcelain surfaces and could be eluted by scrubbing in the presence of an eluent such as Tween 80. When the toilet was flushed after the Tween 80 treatment, almost all of the bacteria were removed from the bowl water

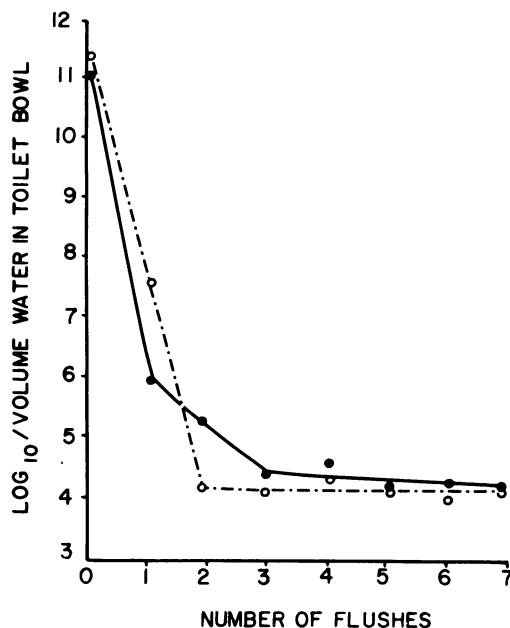


FIG. 1. Effect of flushing on removal of exogenously added bacteria and viruses to the toilet bowl. The  $\log_{10}$  number of organisms indicated above was added to the toilet bowl water, and water samples were removed from the bowl for assay after each flush.  $\log_{10}$  values of organisms represent the number present in the entire volume contained in the toilet bowl. Symbols: ●, MS-2 phage; ○, *E. coli*.

(Fig. 2). In other experiments it was found that simple mixing of Tween 80 into the bowl water or scrubbing the bowl with a brush without addition of Tween 80 were equally efficient in the removal of bacteria from the sides of the toilet bowl.

When the same experiment was performed with poliovirus or MS-2 phage (Fig. 3), an increase in the number of viruses was also noted after Tween 80 treatment, but subsequent flushing resulted in only a gradual loss of virus from the bowl water. Thus, it did not appear that all of the viruses were eluted from the bowl surfaces by the Tween 80 treatment. Perhaps viruses are more difficult to desorb from the porous surface of the porcelain than bacteria.

To determine the number of bacteria usually present in toilets, toilet bowls were monitored in restaurants, service stations, etc. Toilets were treated with Tween 80 and agitated to elute bacteria from the bowl surfaces. A sample of bowl water was then removed and plated on both EMB agar and Trypticase soy agar. The results of these experiments are shown in Table 1. Aerobic bacteria were present at levels from

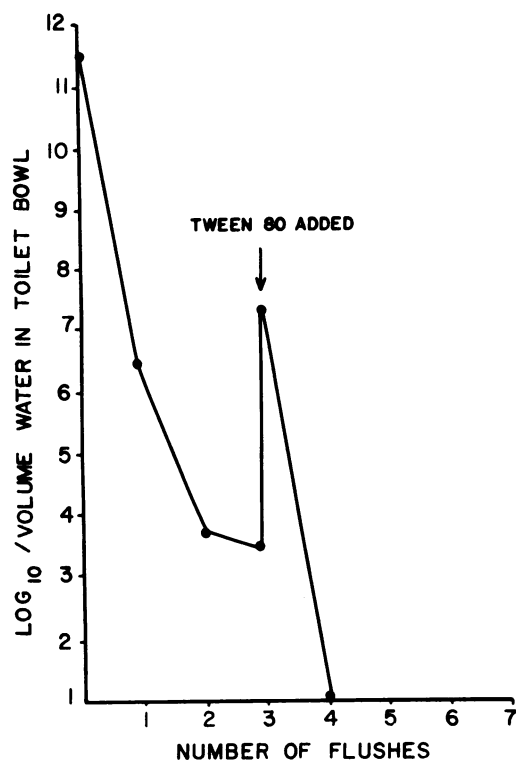


FIG. 2. Elution of *E. coli* from toilet bowl surfaces by addition of Tween 80. Same methods as in Fig. 1, except Tween 80 was added to the bowl water after the flush indicated and the bowl was scrubbed with a brush.

$10^6$  to over  $10^8$  in the bowls tested.

**Droplet production.** Dye studies were performed to determine if water droplets ejected into the air during flushing reach the seat level of the bowl. Crystal violet dye was added to both the bowl and the tank water, and after covering the bowl with a sheet of white absorbent paper the toilet was flushed. By examination of the paper the number of visible droplets produced during flushing was determined. Tank-type toilets produced a random pattern of droplets on the paper, ranging in number from 27 to 104 during a given flush. Valve-type toilets produced fewer visible droplets (between 7 and 10), which were always found towards the rear of the bowl. It appeared that the high pressure of the water coming into the bowl in this type of toilet caused the droplets to be ejected to the rear.

If the bowl was first seeded with *E. coli* and EMB agar plates were exposed at the seat level (taped to a support and facing the bowl water), coliform colonies appeared on the agar plates in

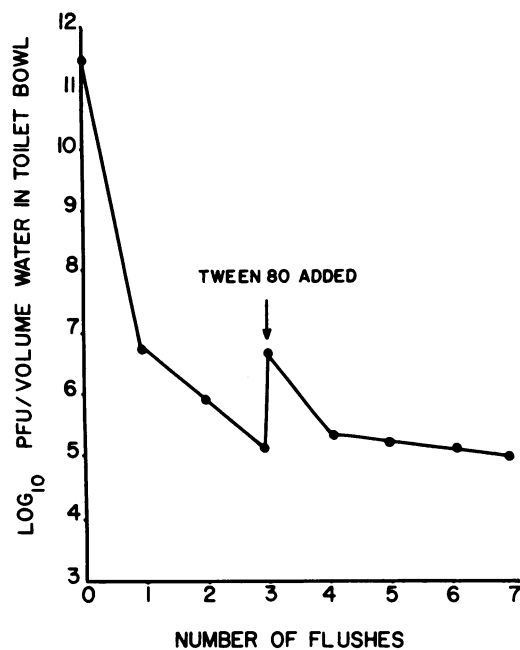


FIG. 3. Elution of MS-2 phage from toilet bowl surfaces by addition of Tween 80. Same methods as in Fig. 2.

TABLE 1. Recovery of bacteria from toilet bowls<sup>a</sup>

Sample no.	Type of establishment	Type of toilet	Total no./vol in toilet bowl (log <sub>10</sub> )	
			Coli-forms	Total bacteria
1	Hamburger stand	Tank	2.50	6.90
2	Service station	Tank	5.50	8.05
3	Hospital	Valve	5.40	7.50
4	Restaurant	Tank	3.10	6.65
5	Motel	Valve	3.90	9.25
6	Service station	Tank	2.50	7.50
7	Home	Tank	3.30	8.25
8	Research institute	Valve	3.70	6.80
9	Restaurant	Valve	8.10	9.30
10	Research institute	Valve	6.10	6.65
11	Research institute	Valve	2.28	7.10
12	Research institute	Valve	3.10	6.80
13	Service station	Valve	4.50	9.40
14	Hospital	Valve	2.25	6.00

<sup>a</sup> Toilets were treated with 220 ml of 1% Tween 80 to remove adsorbed bacteria before sampling.

the same pattern as the droplets.

**Collection of ejected organisms with gauze.** To determine more quantitatively the number of organisms which reach the seat level during

flushing, cotton gauze was utilized to collect ejected organisms. A series of experiments was first performed to determine the efficiency with which bacteria and virus entrapped by the gauze could be recovered. Suspensions of either bacteria or viruses were added to cut squares of gauze (6 by 6 inches [ca. 15 by 15 cm], two thicknesses, 12 actual layers of gauze), and various solutions were evaluated for their ability to elute the organisms. Gauze pretreated with TSB was found to give the best recoveries of *E. coli* and MS-2, and the average percentage of recoveries for several experiments is shown in Table 2. It has been shown that enteric viruses adsorbed to a variety of surfaces can be eluted at high pH (17). Thus, glycine buffer adjusted to pH 11.5 was used to elute poliovirus from gauze pretreated with glycine. The average recovery using this method to elute poliovirus from the gauze was 84% (Table 3). With poliovirus it was necessary to concentrate the virus from the gauze eluate before assay. This was accomplished with adsorption onto membranes (Millipore Corp.) as described by Wallis et al. (21).

The following procedure was used to determine the number of organisms reaching the seat level in droplets. The toilet bowl and rim were

first sterilized by igniting alcohol placed on the rim, and the bowl was seeded with the test organism. The bowl was then covered with a piece of gauze (14 by 17 inches [ca. 35.6 by 43.2 cm], 12 thicknesses, double layer) presoaked with 50 ml of TSB or glycine buffer, and the toilet was flushed. The gauze was then soaked for 5 min in 150 ml of eluent with occasional squeezing of the gauze to obtain a maximal amount of eluate. Approximately 100 ml of eluate was usually obtained. The number of bacteria or viruses in the eluate was then quantified. EMB agar placed on top of the gauze held over the bowl indicated that *E. coli* bacteria did not penetrate the gauze after flushing. Table 4 shows the number of *E. coli* ejected from two tank-type toilets with different volumes and the amount of variation in the number of organisms recovered from replicate experiments.

The number of bacteria and phage ejected from the toilet during a flush was found to be directly proportional to the amount present at the time of the flush (Fig. 4). Studies with poliovirus (Table 5) indicated that similar quantities of this virus were ejected from the bowl as found for MS-2 phage when the bowl was seeded with  $10^8$  plaque-forming units. When the number of seed organisms approached numbers found in human stool (about  $10^{12}$  for bacteria [18] and  $10^8$  for virus [16]), as high as  $6.6 \times 10^4$  coliforms and  $2.8 \times 10^5$  plaque-forming units of virus were recovered from the gauze.

The number of coliforms and the total number of aerobic bacteria recovered from the gauze

TABLE 2. Elution of *E. coli* and MS-2 phage from gauze with TSB<sup>a</sup>

Organism	Pretreatment of gauze prior to addition of test organism	No. of organisms placed on gauze ( $\times 10^4$ )	No. of organisms eluted from gauze ( $\times 10^4$ )	% Recovery
<i>E. coli</i>	TSB	19.0	19.5	104
		19.0	20.0	106
		5.0	4.76	95
		5.0	5.56	113
		5.0	4.90	98
<i>E. coli</i>	None, dry	5.0	7.76	15
		5.0	5.95	12
		19.0	7.00	36
MS-2	TSB	117	106	90
		117	89	76
		117	92	79
		117	80	68
		117	91	78

<sup>a</sup> Gauze pads (6 by 6 inches, 2 thicknesses, 12 actual layers of gauze) were used dry or wetted with 5 ml of TSB. Excess fluids were expressed, and then 0.1 ml of the indicated organisms suspended in tapwater was placed onto the gauze. After 5 min the gauze was soaked in 20 ml of broth, and approximately 7 ml of eluate was expressed from the gauze.

TABLE 3. Elution of poliovirus from gauze pads with pH 11.5 glycine buffer

Gauze pad no. <sup>a</sup>	PFU added to gauze ( $\times 10^5$ )	PFU detected in eluate ( $\times 10^5$ )	% Recovery
1	4.00	3.40	85.0
2	4.00	3.90	97.5
3	4.00	3.00	75.0
4	4.00	3.20	80.0

<sup>a</sup> Gauze pads (4 by 7 inches, 2 thicknesses, 12 actual layers of gauze) were wetted with 5 ml of pH 11.5 glycine buffer, and 0.1 ml of LSc poliovirus type 1 suspended in tapwater was placed onto the gauze. After 5 min the gauze pads were soaked in 20 ml of pH 11.5 glycine buffer for another 5 min, after which 20 ml of eluate was squeezed from the gauze, and the pH was immediately adjusted to 7.5. PFU, Plaque-forming units.

TABLE 4. Number of *E. coli* ejected from toilets during flushing<sup>a</sup>

No. of bacteria added to toilet bowl ( $\times 10^6$ )	No. of bacteria ejected <sup>b</sup>
<b>Experimental toilet 1</b> (bowl volume, 3,500 ml)	
9.97	1,260
9.97	1,095
15.70	1,340
105.0	5,500
157.0	3,040
245.0	66,500
<b>Experimental toilet 2</b> (bowl volume, 5,900 ml)	
44.8	1,170
21.8	1,925
37.7	2,440
318.0	1,700
171.0	9,000
283.0	2,630

<sup>a</sup> Both toilets had tank volumes of 21,000 ml.

<sup>b</sup> Represents number of bacteria collected from gauze pad held at seat level over the bowl during flushing.

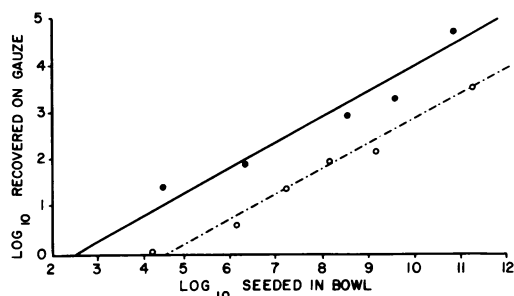


FIG. 4. Concentration of bacteria and virus in toilet bowl and numbers ejected during flushing. The  $\log_{10}$  number of *E. coli* or MS-2 phage indicated above was placed in the bowl water, a gauze wetted with TSB was placed over the bowl, and the toilet was flushed. The  $\log_{10}$  of the number of organisms recovered from the gauze is indicated in the ordinate of the above figure. Symbols: ●, MS-2 phage; ○, *E. coli*.

when actual fecal material was present are shown in Table 6. These data indicated that numbers of bacteria reaching the seat level of the bowl were not appreciably different when the bowl was seeded with similar numbers of bacteria as cultures, homogenized stool, or as a solid fecal pellet. The apparent increase in the ratio of total bacteria to coliforms may be the result of the use of selective media in the assay of the coliforms; i.e., bacteria may be damaged during aerosolization and fail to grow on the selective media.

TABLE 5. Number of polioviruses ejected from toilet during flushing

No. of PFU added to toilet bowl ( $\times 10^6$ )	No. of PFU detected in eluate	Estimated no. of PFU present in eluate <sup>a</sup>
2.88	1,570	2,802
2.67	675	1,205
3.78	300	536
2.94	893	1,594

<sup>a</sup> To determine efficiency of concentration, a portion of the baseline sample was added to a volume of pH 11.5 glycine buffer and concentrated by the same method as the eluate. Estimated number of plaque-forming units (PFU) was calculated as follows: actual number of viruses collected by gauze = (100/% efficiency of elution from gauze) (100/% efficiency of concentration from eluate) (number of viruses detected in eluate). Efficiency of elution from gauze, 84%; average efficiency of concentration using this method, 67%.

TABLE 6. Number of bacteria ejected from toilets during flushing using human stool

No. of bacteria present in toilet water at time of flush ( $\times 10^6$ )		No. of bacteria ejected <sup>a</sup>	
Coliforms	Total	Coliforms	Total
<b>Homogenized stool</b>			
0.182	2.8	10	4,500
1.19	1.54	35	2,000
6.47	7.0	35	4,000
7.35	12.9	60	18,000
18.20	28.2	38	8,000
<b>Solid stool</b>			
0.0007	0.0035	2	6,000
1.01	9.10	85	137,000
0.168	2.30	145	10,000
0.178	1.68	30	21,000
1.53	3.06	280	7,000
<b>Control<sup>b</sup></b>			
0	0	0	2,500
0	0	0	4,500
0	0	0	710
0	0	0	1,090

<sup>a</sup> Represents number of bacteria collected from gauze pad held at seat level over the bowl during flushing.

<sup>b</sup> Gauze was placed over the bowl, but the toilet was not flushed. Organisms detected in the controls probably represent those naturally present in the bowl water or from contamination during collection of the eluates.

**Fallout of airborne *E. coli* onto bathroom surfaces.** To determine if bacteria ejected into air from the bowl during flushing were falling out onto surfaces in the bathroom, EMB agar

plates were exposed at various times after a toilet seeded with *E. coli* had been flushed. The design of the experimental bathroom can be seen in Fig. 5. Agar plates were placed at 6-inch (ca. 15-cm) intervals throughout the room for a total number of 50 plates exposed at one time. Four sets of plates were usually exposed 2, 4, and 6 h after a flush, plus a control set exposed 2 h before the toilet was flushed. The results of these experiments are summarized in Table 7. Within the first 2 h, bacteria were usually detected only in a limited area around the toilet, whereas bacterial colonies detected at later intervals were more randomly distributed throughout the room.

To detect the fallout of airborne particles containing viruses from flushed toilets, 10- by 8.75-inch (ca. 25.4 by 20.9 cm) squares of gauze mounted on metal screens and wetted with TSB were placed at the locations shown in Fig. 5. At the end of the exposure time (each set of gauze was exposed at 2-h intervals), as much fluid as possible was expressed from the gauze and assayed for virus. Since it was desirable to keep the amount of elution fluid to as small a volume as possible, a series of experiments was conducted to determine the effect of eluate volume on the efficiency of virus recovery. The results of these experiments are shown in Table 8. When the eluate volume was less than 10 ml, the efficiency of virus recovery was appreciably reduced. Thus, enough TSB was added to the gauze so that the eluate volume did not fall below this amount.

The results of fallout experiments using MS-2

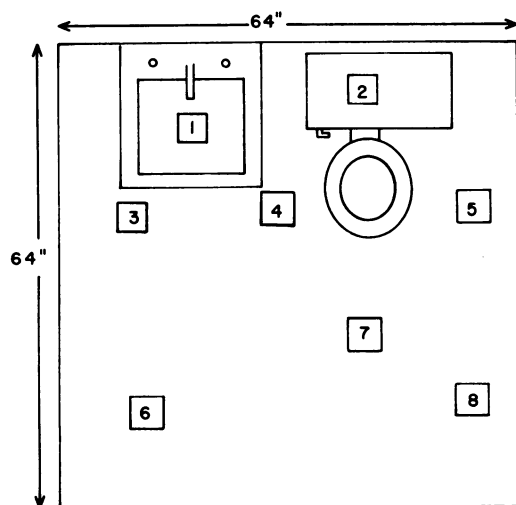


FIG. 5. Floor plan of experimental bathroom with location of gauze pads for viral fallout experiments.

TABLE 7. Number of bacteria detected after falling out on bathroom surfaces after flushing

Time after flush (h)	Avg no. of plates on which bacteria grew <sup>a</sup>	Avg no. of colonies detected per flush	Estimated no. of bacteria on floor surface area of bathroom <sup>b</sup>	Ventilation <sup>c</sup>
0-2	7.0	9.0	737	Closed
2-4	2.8	3.0	246	Closed
4-6	0	0	0	Closed
0-2	7.8	9.2	639	Open
2-4	2.0	2.0	164	Open
4-6	0	0	0	Open

<sup>a</sup> Represents the average of six experiments for each 2-h period. In each experiment  $10^{11}$  *E. coli* were added to the bowl before flushing. Fifty EMB agar plates, mainly on the bathroom floor, were exposed during each 2-h period and replaced by another set of plates.

<sup>b</sup> Calculated as follows: (total floor surface area of bathroom/total surface area of all agar plates exposed) (total number of colonies on agar plates).

<sup>c</sup> The ventilation of the room was considered closed when all air vents in the room were sealed and the bathroom door remained closed.

TABLE 8. Effect of eluate volume on recovery of MS-2 phage from gauze pads<sup>a</sup>

Vol of wetting fluid (ml)	Avg vol of eluate (ml)	Avg % recovery of virus
35	11.3	72
30	10.1	78
25	5.0	59
22	4.2	46

<sup>a</sup> Gauze pads (10 by 8.75 inch) were wetted with the amount of TSB indicated above. Virus was added as two drops of 0.05 ml each. After 15 min as much fluid as possible was expressed from each of the gauze pads. This eluate was then assayed for virus.  $1.2 \times 10^8$  to  $6.0 \times 10^8$  viral plaque-forming units were added to each gauze pad.

phage are shown in Table 9. As was observed for the bacteria, most of the virus appeared to fall-out within 2 h after the flush. However, small numbers of virus were often detected on control sets of gauze exposed before the toilet flush, indicating that virus from experiments performed several days previously was still present in the room. These background counts were subtracted from those obtained after each toilet flush.

**Natural contamination of bathroom surfaces by coliforms.** Using 2.5-inch (6.4-cm) diameter Rodac plates of Levine EMB agar, various surfaces in a number of household and

TABLE 9. Number of MS-2 phage falling out on bathroom surfaces after toilet flushing

Time after flush (h)	Total no. of PFU eluted from all gauze pads <sup>a</sup>	Total PFU after correction for efficiency of elution <sup>b</sup>	Estimated no. of PFU on floor surface area of bathroom	Ventilation <sup>c</sup>
0-2	4,107	5,175	35,468	Closed
2-4	244	307	2,532	Closed
4-6	0	0	0	Closed
0-2	209	263	1,540	Open
2-4	0	0	0	Open
4-6	0	0	0	Open

<sup>a</sup> Represents the average of five to six experiments for each 2-h period. In each, approximately  $4 \times 10^8$  plaque-forming units (PFU) of MS-2 phage were added to the bowl before flushing. Eluate from eight gauze pads was pooled before assay. The location of the gauze pads in the bathroom can be seen in Fig. 5.

<sup>b</sup> The average efficiency of elution was approximately 79%.

<sup>c</sup> The ventilation of the room was considered closed when all air vents in the room were sealed and the bathroom door remained closed.

TABLE 10. Detection of naturally occurring coliforms on bathroom surfaces<sup>a</sup>

Surface	No. of samples	No. of agar plates on which coliforms were detected	% Samples positive	Maximum no. of coliforms detected on a single plate
Walls	54	11	20	5
Floor	120	31	26	>100
Seat, toilet	70	27	38	15
Rim, toilet	35	10	28	>60
Flush handle	9	1	11	2
Bathtubs, sinks, cabinets, etc.	125	6	5	>100

<sup>a</sup> Rodac plates of EMB agar were used.

public bathrooms were sampled for the presence of coliforms. The results are summarized in Table 10. Over 20 bathrooms were tested and coliforms were detected in all, indicating that surface contamination in the bathroom is common.

## DISCUSSION

Considerable numbers of bacteria and viruses were shown to remain in the bowl water after

flushing, and even continual flushing could not remove a persistent fraction. This was attributed to the adsorption of the organisms to the porcelain surfaces of the bowl, with gradual elution occurring after each flush. This adsorbed fraction could explain the heavy bacterial aerosols detected by Darlow and Bale (6) after the second flush of toilets seeded with bacteria. In toilet bowl waters tested by us, chlorine was usually absent or was present in very small amounts (<1 mg/liter), probably due to its rapid loss to the atmosphere. In addition, organic matter in the stool would combine with any free chlorine that might be present. Thus, a gradual build-up of both bacteria and viruses in toilets could occur during regular use.

Droplets produced by flushing toilets were found to harbor both bacteria and viruses placed in the toilet before flushing. The average human stool weighs approximately 100 g and contains a total of about  $10^{12}$  bacteria (18), of which  $10^{10}$  or more are coliforms (19). In infected persons, up to  $10^{11}$  *Salmonella* (19) and  $10^8$  to  $10^{11}$  *Shigella* (14) have been detected in the stool. Concentrations as high as  $10^{10}$  *Salmonella paratyphi* B per g of feces have been detected in carriers (19). The number of polioviruses present in the stool of infected persons can be as high as  $10^6$  per g of feces, giving a total of  $10^8$  in the stool (16). For these values, the number of infectious organisms ejected from the bowl would range from 1,000 to over 10,000 based on data obtained in this study.

Organisms collected on the gauze probably represent those contained in the larger-sized droplets that quickly settle out on surfaces in the bathroom and do not take into account the organisms present in smaller droplets which may be airborne for considerable lengths of time. The detection of coliform bacteria and viruses falling out onto surfaces in the bathroom after flushing indicated that these organisms remain airborne long enough to settle on surfaces throughout the bathroom. The number of *E. coli* detected on the agar plates is probably a minimal value since airborne bacteria are damaged during aerosolization and by environmental stresses, making growth on selective media more difficult (13). These data also do not take into account the accumulation of organisms on the walls and other surfaces of the bathroom. The number of viruses calculated to be falling out onto the floor surface of the bathroom was found to be over a log greater than that detected when the gauze was held over the bowl. This may be due to large numbers of virus being present in smaller-sized drops which do not

impact onto the gauze during flushing. Whereas the number of both bacteria and viruses determined to be impacting on surfaces was less when the bathroom door and vents were left open, it should be pointed out that the organisms not settling out in the room under these conditions are probably being carried to other locations by air currents.

The presence of fecal organisms on bathroom surfaces is undoubtedly widespread, as evidenced by the isolation of coliform bacteria on surfaces in all of the bathrooms sampled. Hutchinson (12) detected the presence of *Shigella sonnei* on bathroom floors and toilet seats. He also found that this organism could persist for as long as 17 days on wooden water closet seats. Newsom (14) reported that *Salmonella* survived for 11 days after desiccation when suspended in either tapwater or feces. In this present study, large numbers of coliform bacteria were found on several occasions under shampoo bottles in bathrooms, which might indicate that regrowth or prolonged persistence of these organisms may occur where organic material has a chance to accumulate. In addition, enteroviruses, as well as members of the adenovirus and reovirus groups, have been found to survive desiccation on surfaces (4).

Doses of less than  $10^3$  *Shigella flexneri* have been found to be capable of initiating infection in man (9). Also, Darlow et al. (7) have shown that the lethal dose of *Salmonella typhimurium* in mice was lower when they were infected by inhalation rather than ingestion. With virus, however, the minimal infectious dose for humans may be as low as one tissue culture infectious dose (15). Thus, it would appear that the numbers of bacteria and viruses ejected from the toilet are sufficiently large to initiate infection, especially in the case of viruses.

In a study of airborne transmission of coxsackievirus type 21, Couch et al. (5) detected only 1 50% tissue culture infectious dose per 3.5 ft<sup>3</sup> (0.10 m<sup>3</sup>) in a barracks in which aerosol transmission of the virus between humans occurred. In an army barracks in which transmission of adenovirus was occurring, air sampling revealed the presence of only 1 50% tissue culture infectious dose in 920 ft<sup>3</sup> (25.76 m<sup>3</sup>) of air (2). The results of our study would seem to indicate that greater concentrations of virus would exist as aerosols in bathrooms in which infectious material has been flushed. The spread of viral disease by aerosols from toilets may take on added importance in those viral diseases in which low numbers of viruses are excreted in nasopharyngeal secretions as compared to the amount excreted in the feces. In addition, the

overall importance of enteric virus transmission by respiratory secretions is still in dispute. For example, the spread of poliovirus within families when pharyngeal excretion occurred differed little from that following purely fecal excretion (10). Also, recent studies with adenovirus type 4 indicate that fecal sources may be more important than respiratory sources in the spread of this virus (10).

The significance of enteric virus disease transmission by contact with surfaces harboring infectious material should not be overlooked, as evidenced by the recent findings of Hendley et al. (11) on the transmission of rhinovirus from fomites to the hands and self-inoculation of the eyes and nose. Additional evidence would be necessary to substantiate the role of aerosols in the epidemiology of disease transmission by toilets, but the results of this study indicate that it appears to be a distinct possibility.

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